STUDIES ON A NEW ANTITUBERCULAR DRUG, TRIFLUOPERAZINE: ITS EFFECT IN COMBINATION WITH CONVENTIONAL ANTITUBERCULAR DRUGS IN TUBERCULAR LYMPHADENITIS

R.L. Gupta*, Sundeep Jain*, V.Talwar*, H.C.Gupta* and P.S. Murthy#

Department of Surgery*, Department of Microbiology* and Department of Biochemistry #, University College of Medical Sciences and GTB Hospital, Shahdara, Delhi-110092

ABSTRACT

After demonstrating that trifluoperazine (TFP) possesses *in vitro* antitubercular activity against drug (single and multidrug) resistant *Mycobacterium tuberculosis*, we initiated preliminary clinical studies in a few patients of tubercular lymphadenitis. Effect of TFP was assessed by testing the antitubercular activity of the serum of patients receiving TFP in addition to regular therapy. Patients were divided into two groups of 30 each. For ethical considerations, patients of both groups were treated initially for one month with antitubercular therapy (ATT) consisting of isoniazid, rifampicin, ethambutol and pyrazinamide and TFP was tried for 15 days only. Patients of group1 were given a single dose of TFP (5mg/day) daily from days 31 to 45 in addition to ATT, while those in group 2 received ATT only. Assessment of the antitubercular activity of the serum (tested *in vitro* in Youmans and Karlson's liquid medium) revealed that the serum of patients (collected on 45th day) of group1 (ATT+TFP treated) possessed much higher antitubercular activity than that of group 2 (ATT only treated) patients. Clinical examination indicated that overall improvement was seen much earlier in group1 (ATT+TFP) patients than in group 2 (ATT alone) patients. At the end of the follow up period of 6 months with ATT from 46th day onwards to both groups, there were no side effects due to TFP. Hematology and liver function tests were normal in both the groups. We suggest that TFP has good potential and therefore deserves further studies either in combination with other drugs of ATT or as one of the drugs of ATT, for the treatment of tuberculosis due to MDR strains to find a suitable effective dose without side effects.

KEY WORDS :Trifluoperazine, Antitubercular drug, Tubercular lymphadenitis, Tuberculosis chemotherapy.

INTRODUCTION

For the chemotherapy of tuberculosis, recently Ratnakar and Murthy (1) stressed the need for developing new antitubercular drugs with structures different from those of the existing ones and active against multidrug resistant (MDR) mycobacteria. Based on their biochemical studies Murthy and co-workers (2,3) observed that the

calmodulin antagonist and well known psychotic drug trifluoperazine (TFP) inhibited the *in vitro* growth of drug susceptible and resistant (to one or more drugs) strains of *Mycobacterium tuberculosis* H37 Rv including clinical isolates from tuberculosis patients (4,5). The mechanism of action of TFP also proved to be interesting in that it seems to have multiple sites of action by inhibiting the synthesis of lipids, DNA and proteins from their labelled precursors in *M. tuberculosis*(6). Its structure is different from the antitubercular drugs in use from quite some time like isoniazid, streptomycin, rifampicin, ethambutol, pyrazinamide or the recently developed fluoroquinolone like ciprofloxacin (7). Further TFP possesses the advantage of being in clinical use for psychotic

Author for correspondence

Dr. R.L. Gupta Professor of Surgery at above address or Dr. P.S. Murthy* Editor-in-Chief, Indian Journal of Clinical Biochemistry B164, Sector 14, Noida 20131, UP.

disorders in humans from few decades all over the world. The dosage upto which it is well tolerated and management of side effects, when they occur, are well known to clinicians(8). In view of the above advantages we suggested that TFP in combination with other drugs used for conventional antitubercular therapy has the potential of being a useful new drug for tuberculosis with the advantage of controlling tuberculosis as well as psychic symptoms if present (4,9). Another advantage of TFP is that it has broad spectrum of anti mycobacterial activity. Besides *M. tuberculosis* it inhibits the growth of *M. avium* and viability of *M. leprae* as indicated by ATP synthesis (10,11). However its minimum inhibitory concentration (MIC) when tried alone (2,4) is 4-10 µg/ml, which is high (4,5). But its MIC could be much lower in combination with other antitubercular drugs. We therefore initiated clinical trials in a limited number of tuberculosis patients with a low dose of TFP which is known to be tolerated well without side effects in combination with other antitubercular drugs. The results of our preliminary studies presented here are in patients with tubercular lymphadenitis, which is also prevalent. Overall clinical improvement and antitubercular activity of the serum of treated patients was assessed.

MATERIALS AND METHODS

Sixty patients attending the surgical out patients department, irrespective of age and sex, with the diagnosis of tubercular lymphadenitis, constituted the subjects of study. Due permission from the local ethical committee of our hospital and an informed consent from each patient (parents also in the case of children) were obtained.

Investigations

Clinical, radiological, biochemical, microbiological tests were carried out. Besides general clinical checkup, chest x-ray (in erect), complete hemogram, ESR and platelets count were done. Serum glutamate pyruvate transaminase, alkaline phosphatase, total proteins, albumin and total bilirubin (liver function tests) were estimated by kits from Wipro Biomed, Bangalore. Routine and microscopic examination

of urine was also carried out.

Fine needle aspiration cytology (FNAC) of the lymph node was performed in all patients to confirm the diagnosis and also for the follow up of the patients (12). In cases of multiple nodes, aspirate collected under aseptic conditions was from an affected lymph node nearest to the neck. Air dried smears of the aspirate were stained by Giemsa stain for cytology and Ziehl Neelsen stain for acid fast bacilli (AFB) (13,14). If AFB were present, the aspirate was inoculated on Lowenstein Jensen slopes (14). If FNAC was inconclusive, biopsy of the lymph nodes was performed in patients (15 in Group 1 and 10 in Group 2) who gave consent. The biopsy material was used for histopathology and AFB staining. AFB on the slides were counted and graded according to the criteria of American Lung Association as 0, 1+2+ and 3+. Culture of biopsy material confirmed tuberculosis infections.

Treatment of Patients

Patients were divided into 2 groups of 30 each. For ethical considerations, patients of both the groups were given initially for one month WHO recommended standard antitubercular therapy (ATT). TFP was tried in this preliminary study in one group for 15 days only, that too at a dose of 5mg (per patient) known to be well tolerated without side effects. Group 1 patients received besides ATT additional drug TFP for 15 days from 31 to 45 days and ATT for the remaining period of therapy. Group 2 patients received ATT only through out. ATT consisted of isoniazid 5-10 mg/kg (maximum 300 mg) rifampicin 10mg/kg (maximum 600 mg), ethambutol 25mg/kg and pyrazinamide 30-40mg/kg in 2 divided doses. All the four drugs were given ATT for two months, then isoniazid plus rifampicin for 6-8 months. TFP was Espazine 5 mg tablet of Pharmapak (P) Ltd.

Assessment of the antitubercular activity of the serum of patients treated with ATT or ATT plus TFP

Patients were called fasting to minimize lipemia. They were allowed light breakfast with low fat content and blood was collected and serum was separated within 30-40 minutes. Two blood

samples were collected from every patient one after 30 days (ATT alone but before TFP) and the other after 45 days (either after ATT plus TFP for Group 1 or ATT for Group 2).

RESULTS AND DISCUSSION

All the cases were fresh untreated cases of tubercular lymphadenitis. The age of the patients ranged from 11 to 59 years with a mean age of 22.2 yrs. 32% of the cases were of 11-20 yrs, 40% of 21-30 yrs, 16% of 31-40 yrs, 8% of 41-50 yrs and 4% above 50 yrs. Out of the total patients 46% were males and 54% were females.

Lymph nodes involved were most frequently cervical (78.8%) followed by inguinal (8.9%), axillary (6.7%) and cervical plus axillary (5.6%). The disease at the time of presentation was of 1-39 (mean 6.4) months duration, with 69% of the cases of 1-5 months, 25% of 5 months to 1 yr and the remaining of 1 yr to 39 months duration. Involvement of multiple nodes was in 88% of the cases and single node in 12%. X-ray chest was normal in 90% cases at the time of diagnosis.

Psychotic symptoms like anxiety, depression were seen in many cases in both groups.

ESR was increased and body weight and hemoglobin decreased in nearly 80% of the cases at the time of diagnosis and returned to normal after treatment in both the groups with no statistical difference between them. FNAC was conclusive of tuberculosis in 83% of the cases while in the remaining 17%, histopathological examination confirmed tuberculosis. Examination of the lymph nodes biopsy material revealed that the smear and growth on LJ slopes were indicative of the presence of *M.tuberculosis*. All the biopsy samples which were positive were confirmed to be *M.tuberculosis*.

Assessment of Improvement

General

Fever, malaise, loss of appetite and night sweats were seen in 50-60% of the patients. Fever and loss of appetite showed improvement after a

mean period of 2 and 3 weeks respectively of treatment by ATT alone even before the commencement of therapy by TFP. Dyspepsia caused by ATT treatment was seen in both the groups in 21/30 and 24/30 patients in Group 1 and 2 respectively. TFP helped in better control of the psychotic symptoms when present. ESR which was increased returned to normal value in both the groups without any significant difference in between them. Response of lymph nodes to treatment (regression if there is no fibrosis but reduction in size if fibrosis occurred) was the same in both the groups.

Liver function tests, urine and platelets were normal in all patients at the time of presentation and through out the period of follow up.

Antitubercular activity of the serum of treated patients

For ethical considerations, as explained under methods, patients of both Groups 1 and 2 were treated initially with regular ATT for one month. Since these are preliminary studies to assess the antitubercular activity of TFP in combination with drugs of ATT, TFP was given for 15 days only. A single dose of 5 mg per day was given because it is known to be well tolerated without any side effects (8). Patients in Group 1 received ATT plus TFP while those in Group 2 ATT only from 31 to 45 days. For the remaining period both the groups received ATT only. Therefore the period of assessment of antitubercular activity was with the serum of treated patients collected after 30 and 45 days of treatment i.e. before and after treatment with TFP. The antitubercular activity was tested by adding 0.5ml of serum of treated patients (0.5ml of serum from healthy persons for 3 control tubes) to Youmans and Karlson's liquid medium, in which *M.tuberculosis* was grown (2). The optical density (O.D.) at 540nm of the medium decreased at 45 days when compared with that at 30 days in both the groups (not shown in table 1). However, the decrease in O.D. was much more with the serum of patients in Group 1 than that with Group 2 patients. The O.D. values reflect the growth of *M.tuberculosis* which in turn is an indication of the concentration of antitubercular drugs (or their active metabolites) in the serum which was added to the growth medium. Based on O.D. of 0.48 for

Table 1 Antitubercular activity of the serum of patients treated with ATT plus TFP and ATT alone.

Treatment	Mean O.D. $\pm$ S.D.* (45 days)	%growth reduction (based on control)	No. serum samples with O.D. at 540nm		AFB count in smear of cultures
			0.0-0.3	0.3-0.5	
ATT (30 days) TFP (15 days) (Group1) 24 cases**	0.198 $\pm$ 0.009	59	20	4	0(19) 1+ (5)
ATT (45 days) (Group2) 26 cases**	0.288 $\pm$ 0.009	40	14	12	0(2) 1+(19) 2+(5)
Control (healthy) 3 samples	0.48 $\pm$ 0.01	-	-	all	3+(all)

*O.D. at 540nm of 0.3 corresponds to about 2×10^8 mycobacteria for 5 ml medium under our experimental conditions
 **6 samples in ATT plus TFP group and 4 in ATT group out of 30 samples were contaminated and excluded from the data in this table.

Numbers in brackets in last column indicate the number of samples with particular AFB count.

control tubes (mean of 3 tubes), it appears that the serum of patients treated with ATT alone could reduce O.D. by 40%, but that of the patients treated with both ATT plus TFP could reduce it by 59% (table1). Since growth could be to varying degree with different samples of serum, we analysed the data in a different way also. The number of samples with lesser growth as indicated by lower O.D. of 0-0.3 was much higher (20/24 i.e. 83%) in the ATT plus TFP treated Group1 than in the Group 2 treated with ATT alone (14/26 i.e. 54%). The difference is significant at $P < 0.05$. This interesting observation indicates higher concentration of antitubercular drugs and or their metabolites in the serum of ATT plus TFP treated patients than in the serum of patients treated with ATT alone.

Although O.D. is a quantitative measure of the number of bacilli, it could be argued that there is scope for contamination and O.D. could be due to both mycobacteria and the contaminants put together. As mentioned in table1, six and four samples of Group 1 and Group 2 respectively were contaminated and were excluded from the data in table1. However, in order to be doubly sure, a smear of the medium after taking O.D. after 45 days of growth was made on a slide and stained

with Ziehl Neelsen stain. All the bacilli in all the samples included in table were found to be acid fast mycobacteria (AFB) with out any contamination. Furthermore, analysis of the number of AFB on these slides revealed that AFB count of 0 was seen in 19/24 (79%) cases in ATT plus TFP treated group but only in 2/26 (8%) cases in the group treated with ATT alone. Likewise the number of samples with AFB count of 1+ was only 5/24 (21%) in ATT plus TFP treated group in contrast to a much higher number of 19/26 (73%) in ATT treated group. There were no samples with AFB count of 2+ in ATT plus TFP treated group while 5/26 (19%) had a higher AFB count of 2+ in the ATT treated group. The differences were significant. This clearly conforms that the serum of patients treated with ATT plus TFP had much greater antitubercular activity than the serum of patients treated with ATT alone. This also shows that although the MIC of TFP was higher when tried alone, its antitubercular activity even at a small dose of 5mg when given along with other drugs of ATT was quite good. This is probably due to the fact that not only TFP but also its metabolites might have antitubercular activity.

None of the 30 patients who received TFP complained of any side effects known to be caused

Table 2. Effect of antitubercular therapy on serum proteins

Treatment	Total proteins g/100ml		Albumin g/100 ml		A/G ratio	
	O M	6 M	O M	6 M	O M	6 M
ATT+TFP*	7.62±	7.86±	3.92±	4.19±	1.06±	1.15±
(Group 1)	0.88	0.73	0.50	0.44	0.13	0.15
ATT only	7.87±	7.95±	4.30±	4.56±	1.22±	1.33±
(Group 2)	0.84	0.83	0.59	0.67	0.23	0.27

* TFP was given for 15 days from 31 to 45 days. ATT was given for the remaining period in Group 1 patients while Group 2 patients received ATT only throughout.

Values represent mean± S.D. at the beginning (0 month) and end of the treatment (6 months).

Table 3. Effect of antitubercular therapy on serum glutamate pyruvate transaminase, alkaline phosphatase and bilirubin

Treatment	SGPT		ALP		Bilirubin	
	O M	6 M	O M	6 M	O M	6 M
ATT+TFP*	36.40±	24.7±	328±	339±	0.82±	0.73±
(Group 1)	36.6	12.1	126	94	0.62	0.43
ATT only	34.8±	25.9±	326±	339±	0.71±	0.71±
(Group 2)	34.0	12.4	12.5	95	0.36	0.36

*TFP treatment was as explained under table 2.

by it. Liver function tests (total proteins, albumin A/G ratio Table 2, SGPT, alkaline phosphatase and bilirubin Table 3) were all normal even in the group of patients supplemented with TFP.

In conclusion, supplementation of TFP at a dose of 5mg per day even for a very short duration of 15 days to regular antitubercular therapy conferred the advantage of additional (better) antitubercular activity as judged by overall clinical response especially antitubercular activity of the serum and relief of psychotic symptoms. This also indicates that there is probably either synergism or additive effect between TFP and the other antitubercular drugs tested here. As mentioned above and as indicated by the present results the

advantages of TFP are: (1) its structure is different (phenothiazine) from that of other antitubercular drugs, (2) it inhibits DNA, lipid and protein synthesis of *M. tuberculosis* within 6 hr, (3) it is active *in vitro* against multi drug resistant clinical isolates, (4) it is useful *in vivo* in patients of tubercular (present study) lymphadenitis in combination with other drugs and (5) it is already in use as an anti psychotic drug from a long time. This justifies further studies (1) with TFP 5mg supplemented to ATT from the beginning of treatment itself for a longer time (2) supplementation of TFP at higher doses with ATT to find out maximum tolerated dose from the point of view of efficacy and safety and (3) in cases of tuberculosis caused by multi drug resistant strains.

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